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# THE STATE OF L-CA<sup>2+</sup> CHANNELS IN HYPERTENSIVE VESSELS

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Several reports suggest that altered Ca<sup>2+</sup> handling is associated with the development of hypertension. Abnormalities of Ca<sup>2+</sup> channels of vascular smooth muscle membrane in hypertension are suggested by the observation that arteries from hypertensive rats show increased sensitivity to the Ca<sup>2+</sup> channel activator Bay K 8644 and even to the elevation of extracellular Ca<sup>2+</sup> and show a higher affinity for PN-200110 binding. We have reported that abnormalities of Ca<sup>2+</sup> channels in arteries from hypertensive animals and their lower membrane potential may be related to labile factors which could be vasoconstrictors such as endothelin-1.

We investigated the effect of BQ-123, an antagonist of the ET<sub>A</sub> receptor, which has been reported to produce a significant decrease in blood pressure in stroke-prone spontaneously hypertensive rats and in transgenic renin hypertensive rats on the reactivity of the SHR aorta to the Ca<sup>2+</sup> channel activator Bay K 8644. BQ 123 (1 μM) decreased the sensitivity to Bay K 8644 of aortic rings of SHR down to that of WKY.

This result suggest that endothelin could be involved in the hyperreactivity of Ca<sup>2+</sup> channels in SHR aorta. The effect of BQ-123 cannot be attributed to an interaction with the NO release since the experiments were performed in the presence of L-NNA. We have previously shown that threshold of subthreshold concentrations of endothelin-1, close to the physiological one, can potentiate the responses to vasoconstrictor agents and to Bay K 8644. Significant increase in the immunoreactive endothelin-1 content and in the pre-proendothelin-1 gene expression have been found in vessels from DOCA-salt hypertensive rats suggesting that endothelin could be increased in hypertension. Our observation of the specific action of an endothelin antagonist in isolated SHR aorta is in full agreement with a role of endothelin in the pathogenesis of hypertension. The question open is the mechanism by which the peptide could affect the state of Ca-channels in hypertensive arteries.

## References:

- 1 Godfraind T, Kazda S, Wibo M. Effect of chronic treatment by nisoldipine, a calcium antagonistic dihydropyridine, on arteries of spontaneously hypertensive rats. *Circ Res* 1991; 68: 674-682.
- 2 Morel N, Godfraind T. The endothelin ET<sub>A</sub> receptor antagonist, BQ-123, normalizes the response of the SHR aorta to Ca<sup>2+</sup> channel activator. *Eur J Pharmacol* 1994; 252: R3-R4.
- 3 Morel N, Godfraind T. Selective interaction of the calcium antagonist amlodipine with calcium channels in arteries of spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 1994 (in press).

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# EFFECTS OF ENDOTHELIN-1, BASIC FIBROBLAST GROWTH FACTOR AND ACTIVIN A ON MITOGENESIS AND MITOGEN-ACTIVATED PROTEIN KINASE IN SWISS 3T3 FIBROBLASTS

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Activins are members of a superfamily of peptides that includes the transforming growth factors β, inhibins, bone morphogenic proteins, etc. They are implicated in the regulation of a variety of biological event including the cardiovascular development of embryo. We found that Swiss 3T3 fibroblasts express activin receptor abundantly. In an attempt to characterize the mitogenic action of activin A, we examined the effects of activin A as well as ET-1 and bFGF on the DNA synthesis and the MAP kinase activity, which is thought to play important roles for G0/G1-S transition. All the human recombinant activin A (10 nM), ET-1 (10 nM) and bFGF (5 ng/ml) potently stimulated the [<sup>3</sup>H]thymidine incorporation into DNA. Although ET-1 and bFGF increased MAP kinase activity, activin A at 10 nM did not affect the kinase activity. Furthermore, ET-1 and bFGF, but not activin A did induce the phosphorylation of MAP kinase. These observation suggest that the activation of MAP kinase is not involved in the activin A-induced DNA synthesis.

## References:

- 1 Sakurai T, Abe Y, Kasuya Y, Takuwa N, Shiba R, Yamashita T, Endo T, Goto K. Activin A stimulates mitogenesis in Swiss 3T3 fibroblasts without activation of mitogen-activated protein kinases. *J Biol Chem* 1994, in press.
- 2 Vale W, Hsueh A, Rivier C, Yu J. Handbook of Experimental Pharmacology. Eds. Sporn MA, Roberts AB. Springer Verlag, Berlin. 1990; vol 95: 211-248.
- 3 Thomas G. MAP Kinase by Any Other Name Smells Just as Sweets. *Cell* 1992; 68: 3-6.

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# THE BIOCHEMICAL AND PHARMACOLOGICAL CHARACTERIZATION OF A 36 kDa-MICROFIBRIL-ASSOCIATED PROTEIN FROM BOVINE AORTA

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We have reported here the biochemical and pharmacological characterization of a newly identified microfibril-associated protein of 36 kDa (36 kDa-MAP) from bovine aorta. Using Ca<sup>2+</sup>-dependent affinity chromatography on a synthetic compound (CKA-1303)-coupled Sepharose, we obtained pure form of 36 kDa-MAP. This compound should serve as useful tool for clarifying the physiological roles of 36 kDa-MAP. 36 kDa-MAP remains associated with the membrane fraction in the presence of Ca<sup>2+</sup> and non-ionic detergents and is dissociated by EGTA. In addition, <sup>45</sup>Ca<sup>2+</sup>-autoradiography clearly indicated that 36 kDa-MAP binds Ca<sup>2+</sup>. Calvasculin, a newly identified EF-hand protein, is present abundantly in bovine aorta. This protein bound with 36 kDa-MAP in a Ca<sup>2+</sup>-dependent manner in vitro. A stoichiometry analysis showed that the 36 kDa-MAP bound 2.2 calvasculin eq/mol of protein. Solid-phase binding assay

indicated a preferential affinity of native calvasculin for 36 kDa-MAP among the extracellular matrix proteins, such as collagens I-V and fibronectin, in a  $\text{Ca}^{2+}$ -dependent manner. Partial amino acid sequence of 36 kDa-MAP (total 151 residues) was determined. A search of the NBRF data base revealed that 36 kDa-MAP had no significant level of homology with other proteins. Our results suggest the presence of a novel  $\text{Ca}^{2+}$  messenger system in vascular smooth muscle cells. Further characterization of 36 kDa-MAP, particularly its biochemical function and cDNA cloning, should lead to understanding of its role in structure and function of blood vessel wall.

#### References:

- 1 Watanabe Y, Kobayashi R, Ishikawa T, Hidaka H. Isolation and characterization of a calcium-binding protein derived from mRNA termed p9Ka, pEL98, 18A2, or 42A by the newly synthesized vasorelaxant W-66 affinity chromatography. *Arch Biochem Biophys* 1992; 292: 563-569.
- 2 Watanabe Y, Usuda N, Tsugane S, Kobayashi R, Hidaka H. calvasculin, and encoded protein from mRNA termed pEL-98, 18A2, 42A, of p9Ka, is secreted by smooth muscle cells in culture and exhibits  $\text{Ca}^{2+}$ -dependent binding to 36-kDa microfibril-associated glycoprotein. *J Biol Chem* 1992; 267: 17136-17140.
- 3 Kobayashi R, Mizutani A, Hidaka H. Isolation and characterization of a 36-kDa microfibril-associated glycoprotein by the newly synthesized isoquinolinesulfonamide affinity chromatography. *Biochem Biophys Res Commun* 1993; 198: 1262-1266.

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#### ALL-OR-NONE LIKE CALCIUM RELEASE FROM INTRACELLULAR STORES BY AGONISTS IN SMOOTH MUSCLE CELLS

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Calcium release from the intracellular stores is essential in the initial phase of agonist-induced smooth muscle contraction. We have found that in smooth muscle cells, agonist-induced calcium release occurs in an all-or-none like manner. Single cells isolated from guinea-pig taenia caeci mostly gave no response to 1,000 nM carbachol but full response to 2,000 nM. About a half of cells gave full response to 1,500 nM carbachol, while the remainder did not respond at all. Confocal microscopic study revealed that under agonistic action calcium wave(s) starts at the most sensitive spot(s) in a cell and propagate throughout the cell, thus forming the basis of the all-or-none behaviour. Calcium-induced calcium release (CICR) in the narrow sense does not contribute to this propagation, because ryanodine, which affects only open CICR channels to fix the channels in the open state, exerted no effects during agonist-induced calcium release. Calcium-activated inositol-triphosphate (IP<sub>3</sub>) formation is not the basis of the wave either, because the same amount of IP<sub>3</sub> was formed by agonist even when calcium release response was negligible. The accelerating effect of calcium on IP<sub>3</sub>-sensitive channel is exerted quickly enough to constitute a positive feedback loop during a single agonistic action, and this satisfactorily explains the all-or-none type behaviour.

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#### THE PHYSIOLOGICAL AND PHARMACOLOGICAL FEATURES OF NEUROTRANSMITTER-ACTIVATED NONSELECTIVE CATION CHANNELS (NSCC) IN SMOOTH MUSCLE

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In various types of smooth muscles, openings of Ca-permeable NSCC have been identified in response to neurotransmitters and autotoxins. Here we describe the NSCCs of guinea-pig ileum and rabbit portal vein. Stimulation of the muscarinic receptor in guinea-pig ileum activates single cationic channels of 20-30 pS (mNSCC). mNSCC are permeable to cations, with the sequence of  $\text{Ba} > \text{Ca} > \text{Na} > \text{Li} > \text{Cs} > \text{K} > \text{Mg}$ . Quinine and diphenylamine-2-carboxylates potently block mNSCC. Besides these consensus properties of NSCC, mNSCC seem to possess several unique properties. Voltage-dependence: depolarizations increase the open probability of mNSCC. Ca-dependence: the activity of mNSCC is potentiated by the intracellular  $\text{Ca}^{2+}$  ions. pH-dependence: the activity of mNSCC is incrementally regulated by both the intracellular and extracellular proton concentrations. External divalent cations such as  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Ni}^{2+}$  block mNSCC. The involvement of a pertussis toxin-sensitive G-protein has been suggested in activation of mNSCC. Similar properties have been obtained for the  $\alpha_1$ -adrenergic receptor-activated NSCC in the portal vein. They have a single channel conductance of 25 pS, and are voltage-dependent and sensitive to the blockade by divalent cations.

These results suggest that NSCC in smooth muscle are subject to the regulations of various factors changing dynamically in the physiological environments and may participate in the fine control of  $\text{Ca}^{2+}$  homeostasis of smooth muscle.

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#### ENDOTHELIUM DEPENDENT RELAXING INFLUENCE ON VASCULAR SMOOTH MUSCLE IS IMPAIRED IN THE AORTA OF THE DIABETIC, OBESE MOUSE

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Diabetic obese mice (Umeå ob/ob) show dyslipidemia and elevated plasma glucose and insulin levels. These metabolic changes resemble those of many obese, hypertensive humans ("the metabolic syndrome"). The aim of our study was to examine whether the endothelial vascular control in the obese mice differs from that of the lean controls (Umeå ob/+ or +/+). Isometric contractions were measured in rings from thoracic aortae of the two strains. The  $\text{pEC}_{50}$  values for norepinephrine (NE) responses in rings with intact endothelium were similar for lean and obese mice,  $7.72 \pm 0.16$  and  $7.80 \pm 0.18$ , respectively (mean  $\pm$  SD,  $n=10$  and  $11$ ). However, the maximal contractile response to NE in intact rings from obese mice was  $33 \pm 10\%$  of that obtained in presence of  $0.1$  mM N $\omega$ -nitro-L-arginine ( $n=40$ ) whereas this value for rings from lean mice was only  $12 \pm 5\%$  ( $n=15$ ),  $p < 0.01$ . Precontracted ( $10$  nM NE) intact rings from lean and obese mice relaxed by  $95 \pm 4\%$  and  $65 \pm 20\%$  ( $n=4$ ), respectively, in response to  $10$   $\mu$ M acetylcholine (ACh). The ACh response was abolished by mechanical removal of the endothelium in rings from both strains. The results suggest that endothelial NO